

# Sampling and pheromone trapping for comparison of abundance of *Amyelois transitella* in almonds and pistachios

Charles S. Burks<sup>1\*</sup>, Bradley S. Higbee<sup>2</sup>, David G. Brandl<sup>1</sup> & Bruce E. Mackey<sup>3</sup>

<sup>1</sup>USDA-ARS, San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Avenue, Parlier, CA 93648, USA,

<sup>2</sup>Paramount Farming Company, 33141 E. Lerdo Highway, Bakersfield, CA 93308, USA, and <sup>3</sup>USDA-ARS, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710, USA

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## Abstract

The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), is the primary insect pest of almonds, *Prunus amygdalus* Batsch (Rosaceae), and pistachios, *Pistacia vera* L. (Anacardiaceae), in California, USA. Abundance of *A. transitella* was compared between these crops by examining total and infested mummy nuts collected in trees and on the ground between January and March in 2003 and 2004, and by examining the number of males captured in sticky traps baited with virgin females as a pheromone source during the subsequent growing seasons. There was an 8–9-fold greater density of total mummies (potential hosts) in pistachios compared to almonds. The proportion of mummies infested was not significantly different between the crops in 2003, but significantly more almond than pistachio mummies were infested in 2004. In 2003, the average density of infested mummies per hectare was greater in pistachios than in almonds, but in 2004 the converse was true. Examination of meteorological data did not suggest an explanation for more live *A. transitella* per infested mummy in almonds in 2004. The number of males captured in pistachios was consistently greater than the number captured in almonds, particularly during the second flight in June and July. The number of males captured in sticky traps in the summer was more strongly associated with the total mummy density in the sanitation survey of the previous winter than with the density of infested mummies. We conclude that the overall density of mummy nuts serving as potential oviposition sites prior to the next year's crop has a greater impact on the abundance of *A. transitella* during the growing season and subsequent harvest than does the density of infested mummies. The implications for the ecology and management of this pest species are discussed.

## Introduction

The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), is a primary pest of almonds, *Prunus amygdalus* Batsch (Rosaceae), and pistachios, *Pistacia vera* L. (Anacardiaceae) (Zalom et al., 2005; Bentley et al., 2007), which are often planted in close proximity in the southern San Joaquin Valley of California. *Amyelois transitella* is highly polyphagous (Wade, 1961) and gravid females are capable of interorchard flights (Andrews et al., 1980; Andrews

& Barnes, 1982). It was first recorded in California in 1942 (Wade, 1961), was found infesting walnuts and almonds in northern California around 1960 (Michelbacher & Davis, 1961), and was reported as a primary pest of pistachios in 1978 (Rice, 1978).

Studies conducted in the 1970s compared *A. transitella* activity and damage between mature almonds and 5- to 8-year-old pistachios (Rice, 1978; Andrews & Barnes, 1982). Rice (1978) found more eggs on egg traps in pistachios compared to almonds, and Andrews & Barnes (1982) found greater infestation in parts of a 111-ha pistachio orchard adjacent to mature almond orchard compared to other parts. However, since pistachios were first planted for commercial production in California in 1958 (Rice, 1978)

\*Correspondence: Charles Burks, USDA-ARS, San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Avenue, Parlier, CA 93648, USA. E-mail: charles.burks@ars.usda.gov

and require  $\geq 5$  years to reach economical yields and 12–13 more years for full production (Klonsky et al., 1998), much of the 13 360 ha of pistachios in California in 1978 were not yet bearing. As of 2003, there were 37 200 ha of bearing pistachios in California, with an additional 6 000 ha of trees not yet bearing (USDA-NASS, 2005). Thus, the area planted in pistachios and the average ages of pistachio orchards has changed markedly since the late 1970s, when *A. transitella* activity and damage was previously compared between these crops (Rice, 1978; Andrews & Barnes, 1982).

Sanitation – the removal from trees and destruction of unharvested or ‘mummy’ nuts prior to the onset of moth activity the following spring – has been demonstrated to reduce *A. transitella* damage in almonds (Zalom et al., 1984), and is included in pest-management guidelines for both almonds and pistachios (Zalom et al., 2005; Bentley et al., 2007). One important difference between the two crops is that pistachios are alternate bearing; that is, they produce a heavy crop one year followed by a light crop in the subsequent year (Klonsky et al., 1998). In almonds, percent damage is greater in years when there is poor fruit set compared to the previous year (Zalom, 1986). A previous study using egg traps found fewer *A. transitella* eggs deposited by second flight moths on traps in orchards with fewer mummies in the spring (Sanderson et al., 1989). We are, however, unaware of comparative data on seasonal activity of *A. transitella* between almonds and pistachios other than those of Rice (1978).

There are few data using pheromone traps to compare relative abundance of *A. transitella* due in part to ongoing difficulties in developing an artificial pheromone lure for this species. While the primary component of the sex pheromone has been identified (Coffelt et al., 1979), it alone is not sufficient to attract males to a point source (Leal et al., 2005). Other components have been identified more recently (Leal et al., 2005), but a synthetic pheromone lure was not available at the time of this study and therefore the use of virgin females as a pheromone source (Curtis & Clark, 1984; Burks & Brandl, 2004) was the only practical method of comparing male activity. Egg traps are currently the preferred method of timing insecticide application in these crops. However, since they are affected by both the abundance of alternative oviposition sites and the abundance of females (Rice, 1976), they are less suitable as comparisons of relative abundance between crops.

The objective of this study was to compare abundance of *A. transitella* in these two crops, grown in the same region and often in close proximity. Here we compare estimates of total and infested mummies in almonds and pistachios during the winter with the relative abundance of males in these crops in the subsequent growing season.

## Materials and methods

### Abundance of potential and infested hosts in winter sanitation samples

The almond and pistachio ranches used in this study were owned and managed by Paramount Farming Company, LLC (Bakersfield, CA, USA), and their location codes are used to distinguish research sites. They were within an area of  $65 \times 31$  km ( $119^{\circ}3'W$ ,  $35^{\circ}30'N$  to  $119^{\circ}58'W$ ,  $35^{\circ}47'N$ ) in the southern San Joaquin Valley (Kern County) region of California, USA.

Sanitation data were obtained from 114 blocks from 42 almond ranches and 88 blocks from 33 pistachio ranches from the 201 500-ha area described previously. The blocks were square 64-ha subdivisions of the ranches, and the blocks examined were chosen, because the entire area was planted with almonds or pistachios and all 4-ha sampling units (below) were sampled for both 2003 and 2004. The sum of the area examined was 7 296 ha for almonds and 5 632 ha for pistachios.

Data on density of overwintered nuts (mummies) in the trees and on the ground, and on their infestation by *A. transitella*, were obtained from assessments of sanitation effectiveness performed by Paramount Farming research personnel between January and March of 2003 and 2004, after the completion of sanitation procedures. Blocks were subdivided into 4-ha sampling units. Within each sampling unit, the abundance of mummy nuts from the previous harvest was estimated by arbitrarily selecting eight trees comprising two adjacent trees from four consecutive rows from within this area. Separate estimates were made for nuts in the tree and on the ground. For almonds, sampling units were chosen such that four Nonpareil and four pollenizer variety trees were included. All nuts found on the ground between the eight trees were counted, and then telescoping aluminium poles were used to knock nuts from the trees. Each almond mummy sampled was examined in the laboratory to determine whether it had been infested by *A. transitella*. For pistachios, subsamples comprising pistachio mummies from randomly chosen plots were examined to determine the proportion infested, because of the large sample size.

The data from the 16 4-ha sampling units were used to estimate population intensity of mummies (i.e., total mummies per tree) for each 16-ha block, and were multiplied by planting density for that block to obtain estimates of absolute population density of mummies in trees and on the ground (Pedigo, 1994). The density of mummies per tree was multiplied by the proportion of mummies infested to obtain population density of infested mummies, and this product was multiplied by the planting density to obtain an estimate of absolute density of infested mummies. The

proportion of nuts infested was based on samples  $\geq 60$  mummy nuts with  $756 \pm 57$  (mean  $\pm$  SE) and  $1446 \pm 94$  ground mummies per block dissected in 2003 in almonds and pistachios, respectively, and  $1063 \pm 53$  and  $910 \pm 79$  for almonds and pistachios, respectively, in 2004. The number of tree mummies dissected per block was  $19 \pm 2.5$  (mean  $\pm$  SE) for almonds in 2003, and  $29 \pm 4.0$  and  $21 \pm 2.8$  for almonds and pistachios, respectively, in 2004. No tree mummies were found in pistachios in 2003.

The mummies from a subset of 17–19 of the previously mentioned blocks were examined more intensively to estimate the number of live *A. transitella* per infested mummy. The size of these samples for ground mummies totaled 290 almonds and 716 pistachio mummies from the ground in 2003, and 1 453 almond and 516 pistachio mummies from the ground in 2004. For mummies from the tree canopy, the total sample size was 20 almond mummies in 2003, and 133 almond and 31 pistachio mummies in 2004.

Records of precipitation and daily maximum and minimum temperatures were compared between the 135-day periods from 1 November 2002 and 15 March 2003 (winter 2003) and from 1 November 2003 and 14 March 2004 (winter 2004). Data for stations 5, 54, 125, 138, and 146 were obtained from the California Irrigation Management Information System (CIMIS) (<http://www.cimis.water.ca.gov/cimis/welcome.jsp>). Sums were calculated for precipitation for each station for the two winters, and degree days  $< 5^\circ\text{C}$  ( $\text{DD}<5\text{C}$ ) were calculated using the formulae for heating degree days described in <http://www.vesma.com/ddd/ddcalcs.htm>. The 2 years examined were compared to longer term climatic trend by obtaining daily maximum and minimum temperature and precipitation for the winters of 2003 and 2004 for the US National Oceanic and Atmospheric Administration (NOAA) (<http://www.ncdc.noaa.gov/oa/ncdc.html>) recording station in Wasco, CA, and comparing those data with 30-year normals based on recordings from the same station between 1971 and 2000.

#### Males in traps baited with virgin females

Laboratory-reared virgin females were used as a pheromone source for comparing relative abundance of male moths. Groups of three virgin females were sealed in a mesh bag, which was then suspended from the top of a wing trap (Pherocon IC; Trécé, Adair, OK, USA) as described by Burks & Brandl (2004). Moths were reared from a laboratory colony originally obtained in 1966 from the University of California at Berkeley, and maintained on a wheat bran diet (Tebbetts et al., 1978). We have previously shown that, under mid-summer conditions in central California, *A. transitella* females prepared this way survive and call for 4–6 days, and that 60% of the total trap capture occurs on the 1st night (Burks & Brandl, 2004). Females and trap

liners were replaced and data were collected on a weekly basis. In order to facilitate comparison of these data between crops and years, the beginning of flights (cohorts) was determined based on pheromone and egg trap data (latter not shown).

In 2003, we monitored 256 ha ( $1\,600 \times 1\,600$  m) sites at eight ranches, four of almonds and four of pistachios. The almond ranches were 4–10 years old in 2003 and the pistachio ranches were 30–33 years old, and all were within the area previously described. The distance between each of these ranches and the next nearest location in this study ranged from 3 to 14 km, with a median of 5 km. Groups of nine female-baited flight traps were used to monitor the 256-ha observation block at each of these sites. These traps were arranged in intersecting lines, such that there was a trap in the center of the observation block, and traps 402 and 805 m from the center in each cardinal direction. Data were collected for 37 weeks, from 5 March to 19 November 2003. In 2004, female-baited flight traps were placed in the same arrangement in six of the eight ranches from the previous year to examine male activity in ranches weekly for 14 weeks, from 24 May to 23 August 2004. One almond ranch (3710) was excluded from monitoring in 2004, because it was used for mating disruption experiments, and the closest pistachio ranch (4260) was also dropped.

In 2003, but not 2004, mating disruption research was performed at each of these locations. Each ranch contained four 16-ha ( $402 \times 402$  m) treatment plots centered in each quarter of the ranch. Two (in pistachios) or three (in almonds) of the treatment plots received a mating disruption treatment for *A. transitella* involving release of 105 mg per ha per night of the principal pheromone component. Each of the nine previously described flight traps were 201 to 284 m from the edges of these treatment plots.

#### Statistical analysis

Statistical analysis was done using the SAS system (SAS Institute, 2003). Population intensity of mummy nuts (mummies per tree), absolute population density of mummies (mummies per ha), proportion of mummies infested, and absolute density of infested mummies (infested mummies per ha) were compared using mixed model analysis of variance (ANOVA) (Proc MIXED) with year, crop, and their interaction as fixed effects and ranch within crop as a random effect. To stabilize variance and improve model fit, a log transformation was applied to mummies and an arcsine transformation was applied to the proportion of nuts infested (Sokal & Rohlf, 1995). Degrees of freedom were calculated by the method of Kenward & Roger (1997), and the Tukey adjustment was used for comparison of multiple means. Pearson correlation (Proc CORR) was used to compare season totals

of males captured with density of all and infested mummies at the eight sites described in the previous section. A paired Student's *t*-test was used to compare precipitation (in mm) and  $DD < 5C$  for each CIMIS station between 2003 and 2004.

The association of number of males per trap per week with the position of traps was examined by using contingency table analysis (Agresti, 2007) to compare the proportion of weekly trap counts of 0 between distances from the center of the ranch for each crop–year combination, and between crops for each year. Based on the outcome of that analysis, the mean of the nine traps at each 256-ha block were used as the response variable for repeated measures mixed models ANOVA (Proc MIXED) (SAS Institute, 2003), comparing the number of males captured between almonds and pistachios separately for 2003 and 2004. For these analyses, crop, week, and their interaction were fixed effects; ranch nested within crop was a random factor, and week was a repeated measure using a first-order autoregressive, moving average with ranch nested in crop as the subject. The Bonferroni adjustment was used for comparison of multiple means (Sokal & Rohlf, 1995).

Males captured were compared between years and crops in the six ranches and 14 weeks for which those data were collected both years (26 May to 25 August 2003 and 24 May to 23 August 2004). For that comparison, a repeated measures mixed model ANOVA was used with year, week, crop, and their interactions as fixed effects, and ranch within crop and its interaction with year as random effects. Week was included as a repeated measure using a first order autoregressive moving average with the interaction of year and ranch within crop as the subject, and the Bonferroni adjustment was used for comparison of multiple means. The sums of males captured over the entire monitoring periods in 2003 and 2004 were compared using a one-way ANOVA (Proc GLM). The dependent variable was the sum of males captured for the season for individual traps, the independent variable was the ranch, and the Tukey adjustment was used for multiple comparisons. For each of these analyses, a square root transformation of the weekly mean males per trap was used to stabilize variance.

## Results

### Abundance of potential and infested hosts in winter sanitation samples

There were clearly far more mummies on the ground than in the tree in both crops and both years following sanitation treatments (Table 1). There were significant differences in the population intensity of ground mummies between crops ( $F_{1,73.3} = 61.97$ ,  $P < 0.01$ ) and years ( $F_{1,328} = 13.56$ ,  $P = 0.0003$ ), and the crop\*year interaction was significant ( $F_{1,328} = 122.96$ ,  $P < 0.01$ ). All

**Table 1** Population intensity (nuts per tree, mean  $\pm$  SE) of mummies in the tree canopy and on the ground in winter sanitation assessments of 114 almond and 88 pistachio blocks of 64 ha each

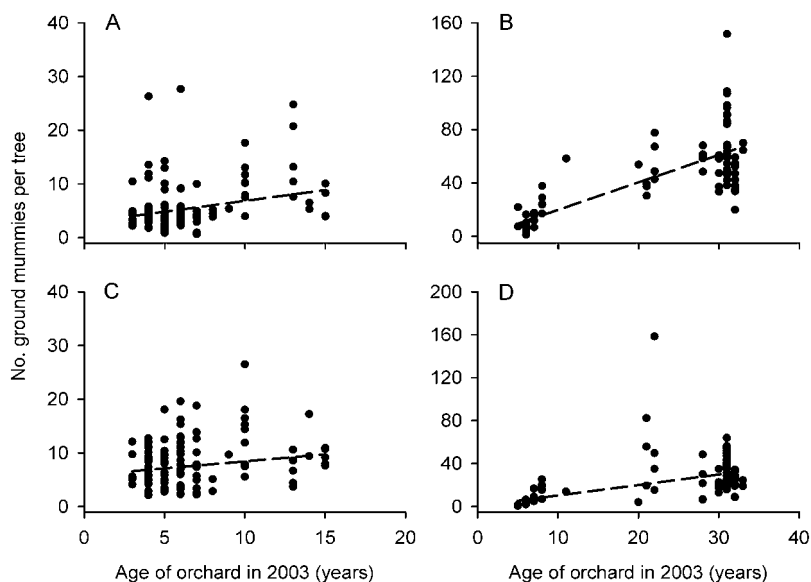
Year	Crop	No. 64 ha blocks	Mummies per tree	
			on ground	in canopy
2003	Almond	114	6.2 $\pm$ 0.45a	0.16 $\pm$ 0.019b
	Pistachio	88	46.6 $\pm$ 3.10d	0.02 $\pm$ 0.004a
2004	Almond	114	8.4 $\pm$ 0.42b	0.23 $\pm$ 0.032b
	Pistachio	88	25.7 $\pm$ 2.37c	0.16 $\pm$ 0.022b

Means within the same column followed by different letters are significantly different (ANOVA:  $P < 0.05$ ).

crop–year means were significantly different ( $P < 0.01$ ) (Table 1). There were significant differences in the population intensity of tree mummies between crops ( $F_{1,88.4} = 7.69$ ,  $P < 0.01$ ), years ( $F_{1,349} = 61.97$ ,  $P < 0.01$ ), and the crop\*year interaction was significant ( $F_{1,349} = 9.31$ ,  $P < 0.01$ ). There were no significant differences in the number of mummies per tree in the canopy between almonds and pistachios in 2004 or almonds in 2003, but there were significantly fewer mummies in the canopy in pistachios in 2003 (Table 1).

The almonds and pistachios examined differed in age: the mean age in 2003 of the almonds was 7 years with a range of 3–15 years old, whereas the mean age in 2003 of the pistachios was 23 years (5–33 years). Within the range of ages examined in the two crops, the number of ground mummies per tree was more strongly associated with age in pistachios than in almonds. There was a significant linear relationship between tree age and mummies on the ground in both crops and both years, but the  $r^2$  values were much higher in pistachios than in almonds (Table 2, Figure 1).

Pistachios and almonds also differed in planting density, with pistachios generally more densely planted. In almonds, the tree density was 214 trees/ha in 51 of the ranches, and the remaining five ranches had densities of 203, 243, 267, 269, and 427 trees/ha. Pistachio densities were more variable; tree density ranged from 275 to 573 trees/ha with a median density of 359. Absolute density of mummies on the ground (population intensity  $\times$  planting density) was 9.7-fold greater in pistachios than in almonds (Figure 2A). For mummies on the ground, there were significant differences in absolute density between years ( $F_{1,328} = 13.56$ ,  $P < 0.01$ ) and between crops ( $F_{1,73.3} = 61.97$ ,  $P < 0.01$ ), and a significant crop\*year interaction ( $F_{1,328} = 122.96$ ,  $P < 0.01$ ). The mean number of ground mummies per ha was significantly different ( $P < 0.01$ ) for each crop–year combination.



**Figure 1** Population intensity (black dots) of ground mummies in (A, C) almonds and (B, D) pistachios in (A, B) 2003 and (C, D) 2004, and regression of ground mummies on tree age (dashed lines).

There were also significant differences in absolute density of mummies in the tree canopy due to both the main effects (crop:  $F_{1,88.4} = 7.69$ ,  $P < 0.01$ ; year:  $F_{1,349} = 36.62$ ,  $P < 0.01$ ), and their interaction ( $F_{1,349} = 9.31$ ,  $P < 0.01$ ).

There were also significant differences in the proportion of mummies infested between crops and years (Table 3). For mummies on the ground, there were significant effects on proportion infested due to year ( $F_{1,327} = 131.77$ ,  $P < 0.01$ ), crop ( $F_{1,73} = 84.69$ ,  $P < 0.01$ ), and their interaction ( $F_{1,327} = 305.91$ ,  $P < 0.01$ ). There was no significant difference in the proportion of ground mummies infested in almond and pistachios in 2003, but there were significantly more almond mummies and significantly fewer pistachio mummies infested in 2004. There were also significant differences in the proportion of infested mummies in trees ( $F_{2,228} = 34.83$ ,  $P < 0.01$ ). There was a significantly smaller proportion of tree mummies infested in almonds in 2003 compared to both crops in 2004, but there was no significant difference in the proportion of tree mummies infested in almonds and pistachios in 2004 (Table 3).

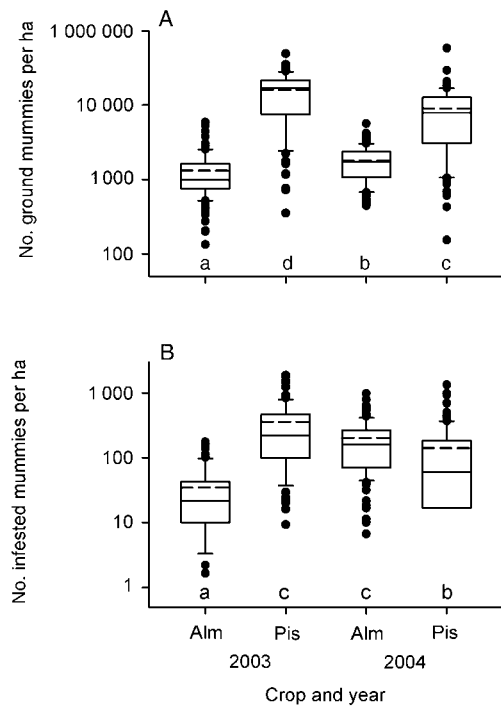
The effect of crop on the absolute population density of infested mummies per ha (both tree and ground) between

crops was not significant ( $F_{1,73} = 0.93$ ,  $P = 0.34$ ), but there were significant differences between years ( $F_{1,327} = 6.86$ ,  $P < 0.01$ ) and the year\*crop interaction was significant ( $F_{1,327} = 354.12$ ,  $P < 0.01$ ). The number of infested mummies per ha was not significantly different between pistachios in 2003 and almonds in 2004, but there were significantly more infested mummies per ha in pistachios than almonds in 2003 and in almonds than pistachios in 2004 (Figure 2B).

The number of surviving *A. transitella* per infested ground mummy did not differ significantly between the crops ( $F_{1,59.7} = 1.55$ ,  $P = 0.22$ ), but did differ significantly between years ( $F_{1,8.96} = 8.07$ ,  $P = 0.02$ ) and the crop\*year interaction was significant ( $F_{1,29.12} = 59.7$ ,  $P < 0.01$ ). The number of live larvae was not significantly different in almonds and pistachios in 2004, but in 2003 this ratio was significantly greater in almonds than in pistachios (Table 4). Estimates of surviving *A. transitella* per infested mummy in trees were necessarily based on much smaller samples, and therefore the ratio of live *A. transitella* per infested tree mummy is reported without analysis (Table 4).

**Table 2** Regression of log transformation of ground mummies per tree on tree age in 114 almond and 88 pistachio blocks at time of winter field sanitation assessments

Crop	Year	Intercept	Slope	F	P	r <sup>2</sup>
Almonds	2003	1.44 ± 0.11	0.057 ± 0.015	13.73	0.0003	0.11
	2004	1.93 ± 0.10	0.029 ± 0.014	4.11	0.0451	0.04
Pistachios	2003	2.01 ± 0.13	0.068 ± 0.005	173.59	<0.0001	0.67
	2004	1.56 ± 0.16	0.060 ± 0.006	90.96	<0.0001	0.51



**Figure 2** Boxplots showing frequency distributions of absolute density of (A) total and (B) infested (total on the ground and in the canopy) mummies in 2003 and 2004 in 114 64-ha blocks of almonds (Alm) and 88 blocks of pistachios (Pis). The whiskers represent 10th and 90th percentile observations, the dots represent individual observations outside this range, the outsides of the boxes represent 25th and 75th percentile observations, and the solid and dashed lines represent the mean and median, respectively. Means with different letters within the same plot are significantly different ( $P < 0.05$ ).

The average precipitation for the five climatic data recording stations was  $111 \pm 18$  mm (mean  $\pm$  SE) in winter 2003 and  $110 \pm 17$  mm in winter 2004; this difference was not significant ( $P > 0.05$ ). The mean accumulation of

DD<5C was  $47 \pm 2.8$  for winter 2003 and  $60 \pm 3.9$  for 2004. This difference was significant ( $t = -4.40$ , d.f. = 4,  $P = 0.01$ ). For the NOAA station in Wasco the 30-year normal precipitation for the 135 days starting from 1 November is 129.3 mm; for winters 2003 and 2004 the accumulation was 99.6 and 98.8 mm, respectively. The 30-year normal accumulation of DD<5C at Wasco is 59.4; for winters 2003 and 2004 the accumulation was 66.5 and 88.6 mm, respectively.

#### Males in traps baited with virgin females

The frequency of empty traps (i.e., 0 male captured at an individual trap in a particular week) differed between distances from the center of the 256-ha block, crop, and year. The center traps were empty significantly more often than others in 2003 in almonds ( $\chi^2 = 21.19$ , d.f. = 2,  $P < 0.01$ ) and pistachios ( $\chi^2 = 6.42$ , d.f. = 2,  $P = 0.04$ ), and in 2004 in almonds ( $\chi^2 = 7.88$ , d.f. = 2,  $P = 0.02$ ). The difference in the proportion of empty traps between almonds and pistachios was different in 2003 ( $\chi^2 = 185.44$ , d.f. = 1,  $P < 0.01$ ) and 2004 ( $\chi^2 = 20.56$ , d.f. = 1,  $P < 0.01$ ).

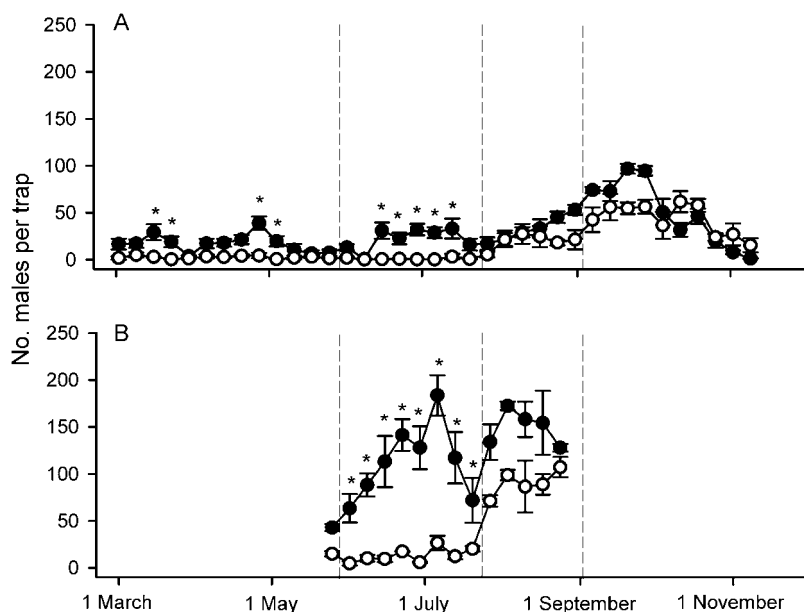
In 2003, there was a significant difference in the number of males captured between the two crops ( $F_{1,9.78} = 26.75$ ,  $P < 0.01$ ), between weeks ( $F_{36,179} = 9.41$ ,  $P < 0.01$ ), and the crop\*week interaction was also significant ( $F_{36,179} = 3.56$ ,  $P < 0.01$ ). Significantly more males ( $P < 0.0014$ ) were captured in pistachios than in almonds in 9 of the 37 weeks examined in 2003, and there was no week in which significantly more males were captured in almonds than pistachios (Figure 3A).

In 2004, there were also significant differences in males captured between crops ( $F_{1,5.25} = 58.62$ ,  $P < 0.01$ ) and weeks ( $F_{13,40.3} = 15.61$ ,  $P < 0.01$ ), and the crop\*week interaction was significant ( $F_{13,40.3} = 5.14$ ,  $P < 0.01$ ). Significantly more males ( $P < 0.0036$ ) were captured in pistachios than almonds in 8 of the 14 weeks examined in 2004, comprising all of flight 2 (Figure 3B). An ANOVA comparing males captured in almonds in 2004 to males captured over the same weeks at the same ranches in 2003 found all main

**Table 3** Percent (mean  $\pm$  SE) of navel orangeworm infestation in mummy nuts on the ground and in trees in 114 almond and 88 pistachio blocks at time of winter field sanitation assessments

Year	Crop	On ground		In tree canopy	
		No. 64-ha blocks	Infested mummies/tree	No. 64-ha blocks	Infested mummies/tree
2003	Almond	114	$2.3 \pm 0.16b$	114	$3.7 \pm 0.68b$
	Pistachio	88	$2.2 \pm 0.02b$	—	—
2004	Almond	114	$11.4 \pm 0.70a$	114	$16.3 \pm 1.63a$
	Pistachio	88	$1.7 \pm 0.15c$	88	$14.5 \pm 2.16a$

Means within the same column followed by different letters are significantly different (ANOVA:  $P < 0.05$ ).



**Figure 3** Male navel orangeworm captured in female-baited flight traps in pistachios (black dots) and almonds (white dots) in 256-ha observation blocks in 2003 and 2004. (A) Mean  $\pm$  SE of 36 traps from four ranches for each crop in 2003. (B) Mean  $\pm$  SE of 27 traps from three ranches for each crop in 2004. Asterisks indicate a significant difference between the means for the two crops (ANOVA:  $P < 0.05$  after Bonferroni correction for multiple comparisons). Dashed vertical lines represent estimated beginnings of the second, third, and fourth flights.

effects were significant (year:  $F_{1,7.94} = 64.15$ ,  $P < 0.01$ ; week:  $F_{13,66.9} = 12.84$ ,  $P < 0.01$ ; crop:  $F_{1,7.94} = 44.55$ ,  $P < 0.01$ ). There were also significant interactions of year\*week ( $F_{13,66.9} = 6.37$ ,  $P < 0.01$ ) and week\*crop ( $F_{13,66.9} = 6.80$ ,  $P < 0.01$ ), whereas the year\*crop and year\*week\*crop interactions were not significant ( $P > 0.05$ ). There were significantly more males ( $P < 0.001$ ) captured in both crops in 2004 compared to 2003 in 12 of the 14 weeks examined, and there were significantly more males captured in pistachios than in almonds in both years in 8 of the 14 weeks examined.

A one-way ANOVA comparing the total males captured over the season, using individual traps as observations, found significant differences between the ranches in 2003 ( $F_{7,64} = 7.14$ ,  $P < 0.01$ ) and 2004 ( $F_{5,48} = 116.94$ ,  $P < 0.01$ ). In both years ranch means fell into numerically separate ranges for the two crops; that is, the pistachio ranch in which fewest males were captured had higher counts than the almond ranch with the most males captured, and in

2004 significantly fewer males were captured in the almond ranch with highest trap activity compared to the pistachio ranch with lowest trap activity (Table 5). In both years, there was greater association of males captured in flight traps with the density of all mummies than with the density of infested mummies (Figure 4).

## Discussion

### Abundance of potential and infested hosts in winter sanitation samples

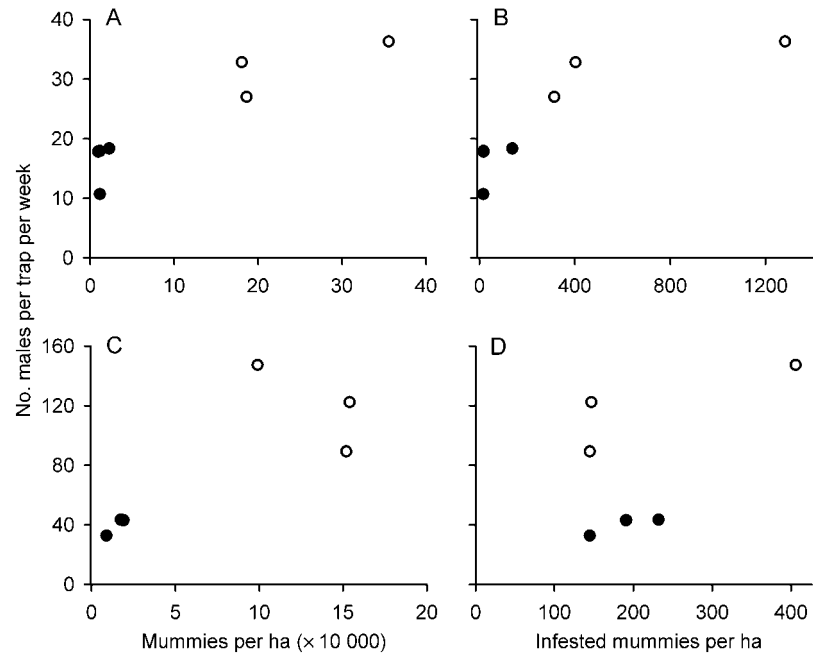
These data clearly demonstrate that, at the time of the winter sanitation samples, there were more mummies in pistachios than in almonds. Under the management used in these ranches, there were far more mummies on the ground than in the tree. The trend we observed of older and more densely planted trees in pistachios compared to almond ranches is typical for the area examined; for instance, cost studies for these crops in this area presume

**Table 4** Number of surviving navel orangeworm per infested mummy (mean  $\pm$  SE) in almond and pistachio mummies sampled from almonds and pistachios ranches at time of winter field sanitation assessments

Year	Crop	On ground		In tree canopy	
		No. 64-ha blocks	Survivors/mummy	No. infested mummies	Survivors/mummy
2003	Almond	17	1.50 $\pm$ 0.129a	20	1.45
	Pistachio	19	0.67 $\pm$ 0.039c	—	—
2004	Almond	18	0.98 $\pm$ 0.106bc	133	2.00
	Pistachio	17	0.99 $\pm$ 0.070b	31	0.87

Means within the same column followed by different letters are significantly different (ANOVA:  $P < 0.05$ ).

**Figure 4** Male navel orangeworms captured in virgin-baited flight traps in the summer in almond (black dots) and pistachio (white dots) ranches vs. estimates of absolute density based on sanitation survey data from the previous winter; trap capture vs. (A, C) density of all mummies and (B, D) infested mummies in (A, B) 2003 and (C, D) 2004. In 2003, Pearson correlation was slightly greater with total mummies ( $r^2 = 0.85$ ,  $P < 0.01$ ) than with infested mummies ( $r^2 = 0.81$ ,  $P < 0.01$ ), whereas in 2004 the difference was more pronounced ( $r^2 = 0.62$ ,  $P = 0.01$  for total mummies, and  $r^2 = 0.17$ ,  $P = 0.42$  for infested mummies).



that almonds are planted at 272 trees per ha and left in place for 22–25 years, whereas pistachios are planted at 359 trees per ha and left in place for 40 years (Beede et al., 2004; Freeman et al., 2008). The slope of the regression of mummies per tree on age was similar between both crops and both years, but pistachio trees are kept longer and the association of mummy population intensity with age is stronger in pistachios compared to almonds. While the greater planting density and greater age of the pistachios compared to the almonds examined contributed to a greater absolute density of mummies in pistachios, the greater relative density indicates that pistachios would have more mummies even in the absence of these factors.

**Table 5** Season total (mean  $\pm$  SE) of navel orangeworm males captured per trap in 2003 and 2004

Crop	Ranch	2003	2004
Almond	3440	657 $\pm$ 69b	603 $\pm$ 35d
	3710	660 $\pm$ 72bc	—
	3740	662 $\pm$ 80bc	608 $\pm$ 39d
	3940	385 $\pm$ 46c	425 $\pm$ 61e
Pistachio	4010	1188 $\pm$ 127a	1160 $\pm$ 69c
	4260	839 $\pm$ 145ab	—
	4510	958 $\pm$ 143ab	2062 $\pm$ 63a
	4840	1299 $\pm$ 166a	1713 $\pm$ 53b

Means within the same year followed by different letters are significantly different (ANOVA:  $P < 0.05$ ).

Because of differences in the production systems, orchard sanitation is inherently more difficult in pistachios compared to almonds (BS Higbee, pers. obs.), and high abundance of pistachio mummies similar to that described in this study was also noted in data collected from pistachios in Madera County, hundreds of kilometers north of the area for this study (Siegel et al., 2008).

The significant difference in the density of mummies between crops indicates that there are more potential hosts in pistachios compared to almonds. However, in this study the proportion of mummies infested was more variable between crops and years. As a result, the average density of infested mummies was more similar between the two crops than the overall mummy density, and was greater in pistachios than in almonds in 2003 whereas the converse was true in 2004. Climate and crop factors do not immediately suggest reasons for variation in the proportion of mummies infested between the crops and years examined. The NOAA data indicate that both the winters of 2003 and 2004 were dry compared to the 30 years from 1971 to 2000, but the CIMIS data indicate there was no difference in precipitation between the two winters. The CIMIS data indicate that the winter of 2004 was colder than the winter of 2003, and the NOAA data indicate that the winter of 2003 was more typical of the 30-year normal. However, since there was a significant increase in the proportion of ground mummies infested in almonds and a significant decrease in pistachios, a general effect of the colder winter in 2004 is not apparent. The overwintered nuts shown here



for the 2003 and 2004 sanitation assessments are from the 2002 and 2003 crops. In pistachios, 2002 was generally a heavy year, and 2003 a light year (USDA-NASS, 2005). There was significantly less infestation of ground mummies in 2004 compared to 2003, the converse of what would be expected if there is generally heavier infestation in a light crop following a heavy one, as previous suggested for almonds (Zalom, 1986). This suggests that, under current conditions the alternate-bearing nature of pistachios has less impact on *A. transitella* abundance and infestation in pistachios than the large amount of potential host material left on the ground after both heavy and light years.

Comparison of the proportion of mummies infested between those sampled on the ground and from the tree suggests greater infestation in tree mummies. This is consistent with the observations of Siegel et al. (2008) in pistachios, but this hypothesis was not examined statistically in this study, because the proportion of infested mummies in trees was necessarily based on a much smaller sample, and because the ratio of mummies in the tree to those on the ground indicates that infested tree mummies were a negligible portion of the overall density of infested mummies per ha.

The number of live *A. transitella* per infested mummy was also variable. In 2004, the number of live *A. transitella* per infested mummy was not significantly different between the crops, and was about one per infested mummy. In 2003, there were significantly more than one *A. transitella* per infested mummy in almonds and significantly fewer than one per infested mummy in pistachios; however, the average 2:1 ratio of *A. transitella* per infested mummies in almonds vs. pistachios is more than compensated for by the average 11:1 ratio of infested mummies per ha in pistachios vs. almonds. Siegel et al. (2008) found substantial mortality between December and the end of February, the period in which the sanitation samples were taken in this study. This mortality would not have affected our estimate of proportion of infested mummies, which included mummies in which *A. transitella* had died or emerged, but it would have affected our estimate of the number of live *A. transitella* per infested nut.

#### Males in traps baited with virgin females

The significant difference in the proportion of weekly counts of 0 male in pheromone traps between positions within both crops in 2003 and almonds in 2004 shows that the nine individual traps in each 256-ha ranch were not independent estimates of relative abundance. A previous study concluded that the range of attraction for *Cydia nigricana* F. was 200 m and the ranges of stimulation were at least 500 m (Wall & Perry, 1987), distances comparable with the intertrap distances at the

256-ha ranches. Another study concluded that traps spaced close enough to mutually interfere should be examined as a unit rather than individually (Elkington & Cardé, 1988), and a different study concluded that inclusion of many observations of 0 can increase type I error when using ANOVA for analysis (Reeve & Strom, 2004). Since the almonds in particular often had traps with 0 moths, and this occurred more in central than peripheral traps, it was therefore most appropriate to pool traps within ranches for the repeated measures analyses that examined differences between crops in individual weeks. When examining the sums of males captured over the entire trapping period, the unit of observation was the individual trap rather than the 256-ha ranch, because, in that case, no traps had counts of 0.

Analyses of both the weekly and whole-season data found males more abundant in pistachios than in almonds. In the weekly analysis, the only statistically significant differences were in periods in which more males were captured in pistachios than in almonds. In 2003 there were 4 weeks, all at the end of the season, in which the mean number of males captured in almonds was numerically (but not significantly) greater than the number captured in pistachios. In 2004, the mean of males captured in pistachios was always numerically greater than the number captured in almonds. The whole-season comparisons in both 2003 and 2004 found that more males were captured in all pistachios ranches examined than in any of the almond ranches. In 2003 all almond ranches and no pistachio ranches had significantly fewer males than the most abundant pistachio ranch; in 2004 all almond ranches had significantly fewer males than any of the pistachio ranches.

A variety of factors are known to affect male response to pheromone sources, including population age structure, presence of conspecific pheromone, mating status of males, and atmospheric conditions (McNeil, 1991). Trap saturation is known to cause a non-linear relationship between the number of males captured and male abundance (Asaro & Berisford, 2001a), and the relationship between temperature and male longevity may effect the relationship between abundance and the number of males captured in pheromone traps (Asaro & Berisford, 2001b). Mating disruption targeting *A. transitella* was in place in each of the ranches examined in 2003, albeit at distances of  $\geq 200$  m from the traps; there was no mating disruption in any of the ranches examined in 2004. There is no a priori reason to believe that any of the aforementioned factors produced a systematic bias that would result in the false impression of greater relative abundance of males in pistachios compared to almonds.

Comparison of male trapping data with sanitation data for the preceding winter indicates a greater association

of relative abundance of males with the total mummy density (potential hosts) than with the winter abundance of *A. transitella* as assessed by the sanitation survey (infested mummies per ha). Zalom et al. (1981) states that sanitation is important both for reducing the number of *A. transitella* that survive to oviposit in the spring and for removing oviposition sites available to the proportion of the population that does survive. Our observations indicate that, under the conditions we examined, the latter function of sanitation is more important than the former.

### Summary and conclusions

We present data from almond and pistachio ranches in Kern County, CA, USA, in 2003 and 2004 indicating that pistachios generally had a greater density of mummies (potential hosts) and greater relative abundance of males, although the absolute density of overwintering *A. transitella* was more variable. We conclude that removal of alternative hosts for oviposition by overwintering and first-generation *A. transitella* before the new crop can support growth (June in almonds, August in pistachios) is a more important function of sanitation than reduction of the overwintering population itself. If so, this makes pistachios, which are harder to sanitize and have a larger abundance of undestroyed mummies, a generally more suitable habitat for *A. transitella* compared to almonds. In well-sanitized almonds, the spring period prior to when the hulls split in the new crop appears to be the greatest bottleneck in population abundance. This also suggests a source-sink relationship between pistachios and almonds in areas where the crops are grown in close proximity. Coordinated management of such meta-populations should benefit producers of both crops.

Limitations in the ability to generalize these conclusions must also be recognized. Kern County, at the southern end of California's Central Valley, is an area of concentrated production of both almonds and pistachios. However, both crops occur in other parts of the central Valley, and there is a climatic gradient with tendency of cooler and wetter winters in the north. The years examined were both dry with respect to long-term climatic norms. Although some moisture enhances the ability to remove mummies from trees, too much moisture makes it more difficult to get into the orchard with the necessary equipment. The pistachio data in this study are partially corroborated by the observations of Siegel et al. (2008) in Madera County, but further studies should examine the relationship between *A. transitella* populations between these two crops in other parts of the central Valley and over a longer period.

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